Severe Acute Respiratory Syndrome (SARS)

Laboratory Diagnosis

Laboratory Assays for SARS

- Detection of virus
  - EM in clinical specimens (CoV-like particles)
  - Isolation of virus
  - Detection of viral antigens (IHC for tissue, ?sensitivity of IFA or ELISA for respiratory specimens)
  - Detection of viral RNA (PCR)
    - Respiratory secretions
    - Stool specimens
    - Urine specimens
    - Tissue – lung and kidney
- Detection of SARS-specific antibody
  - IFA
  - ELISA
  - Neutralization

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Laboratory Diagnosis of SARS Infection

- Type and timing of specimen collection (we need to know more)
- Type of assays
  - Sensitivity
  - Specificity
  - Interpretation of results

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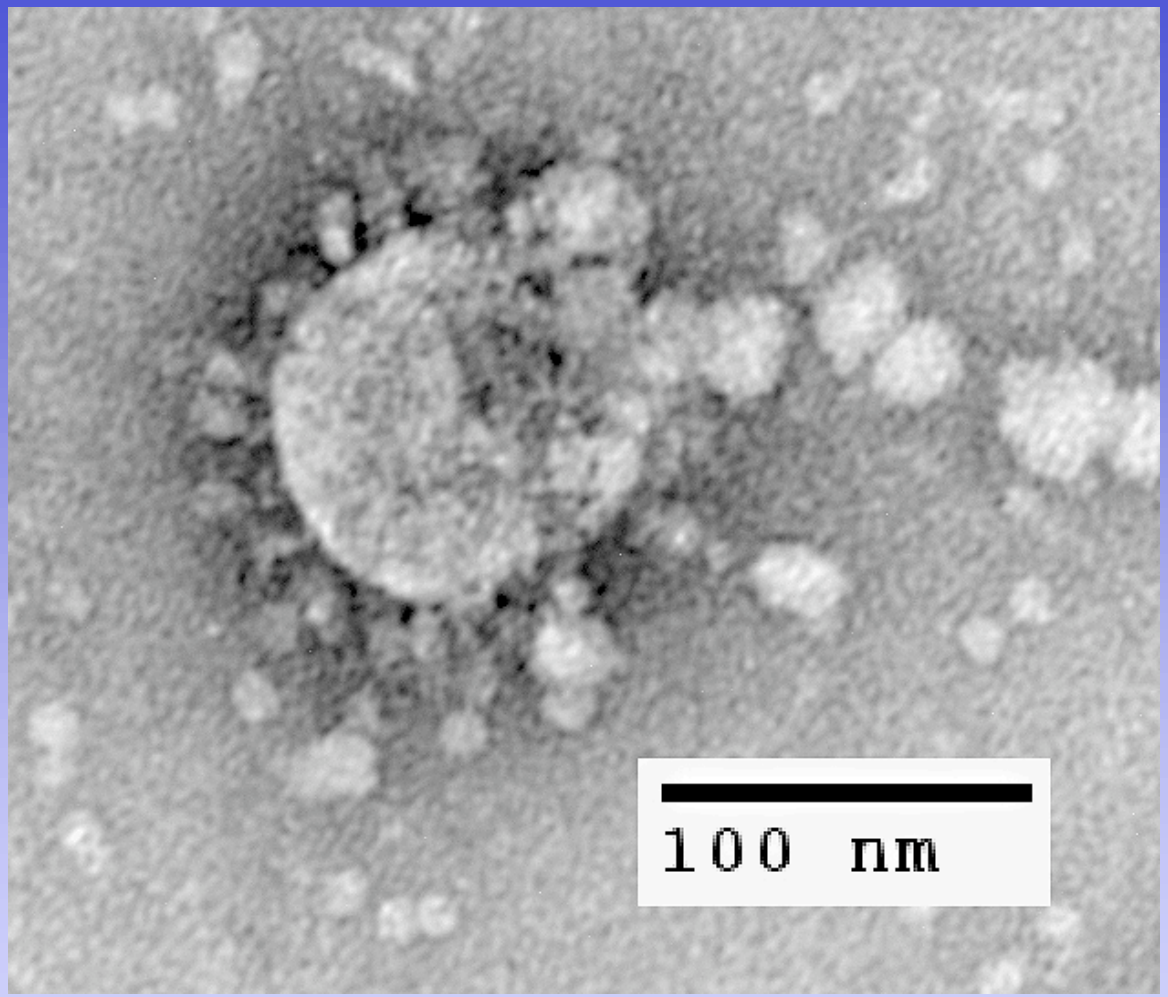

Coronavirus Particle by Negative Stain EM (Isolate From Patient With SARS)

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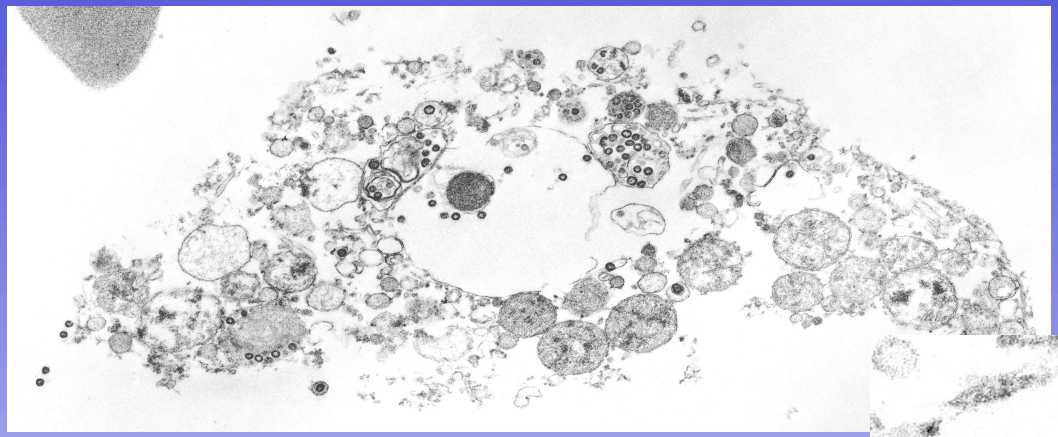
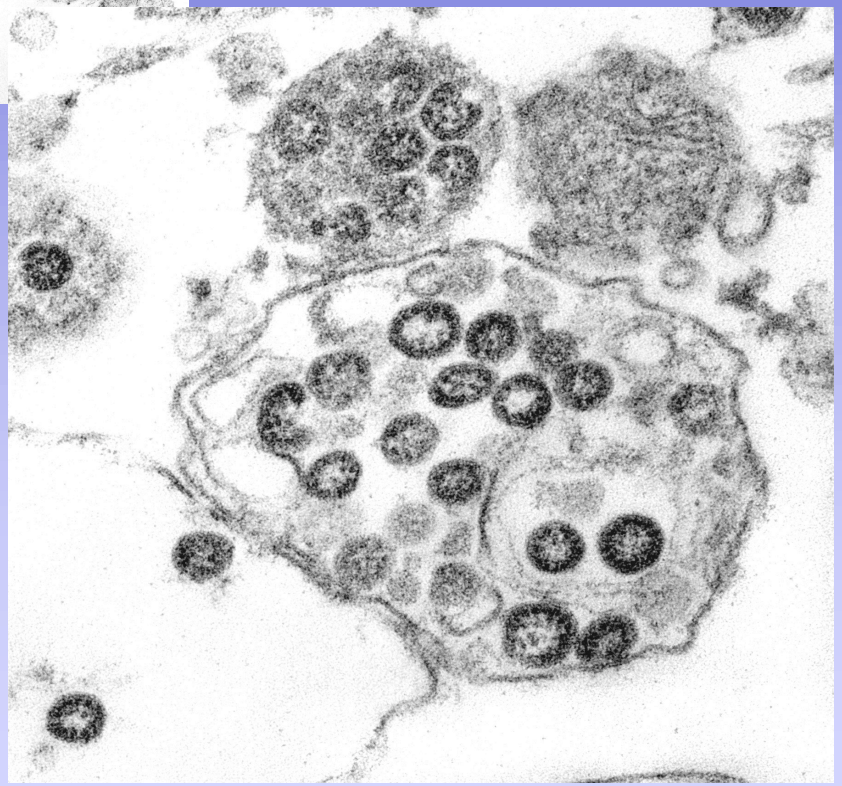
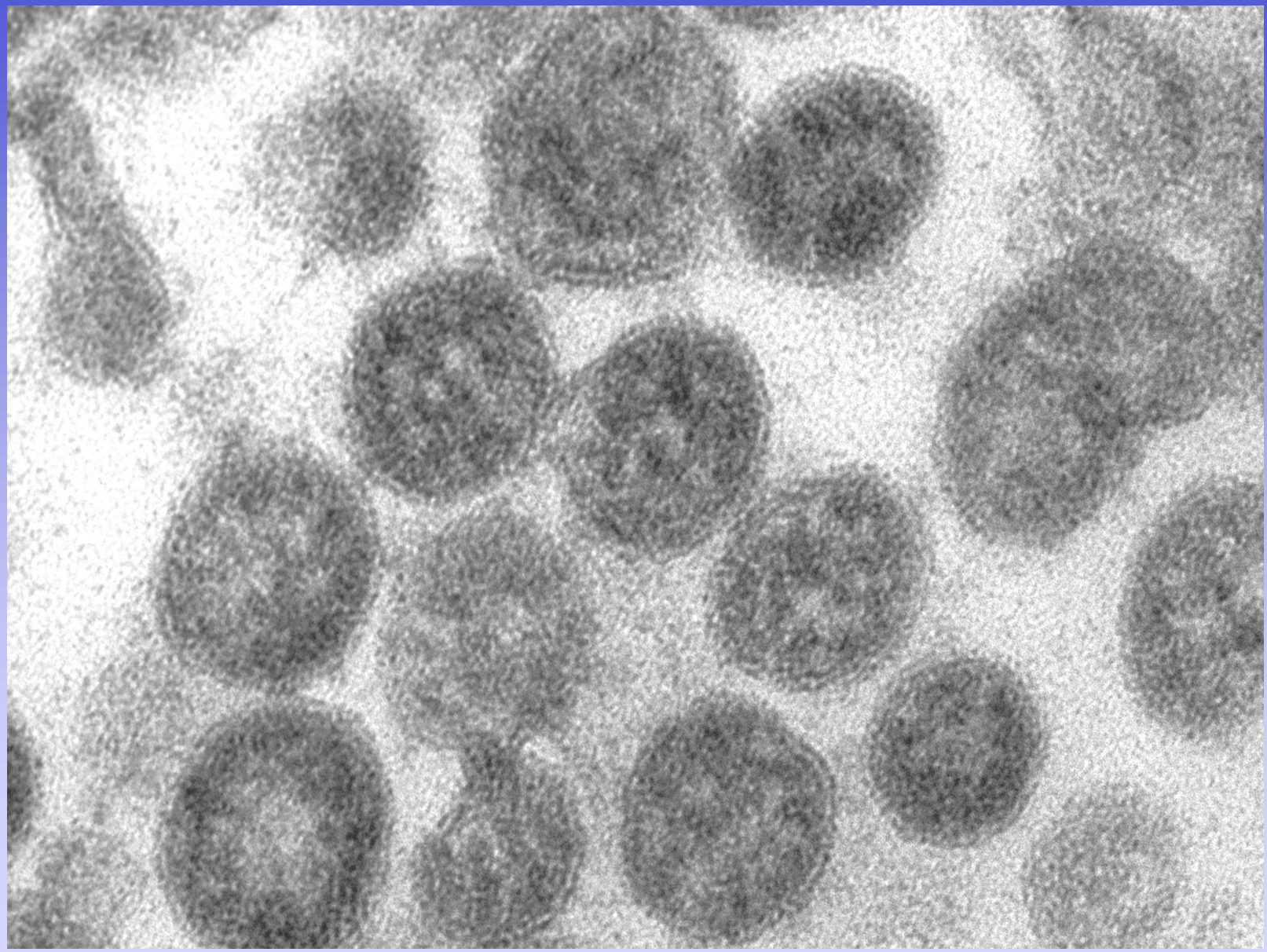


Thin Section EM

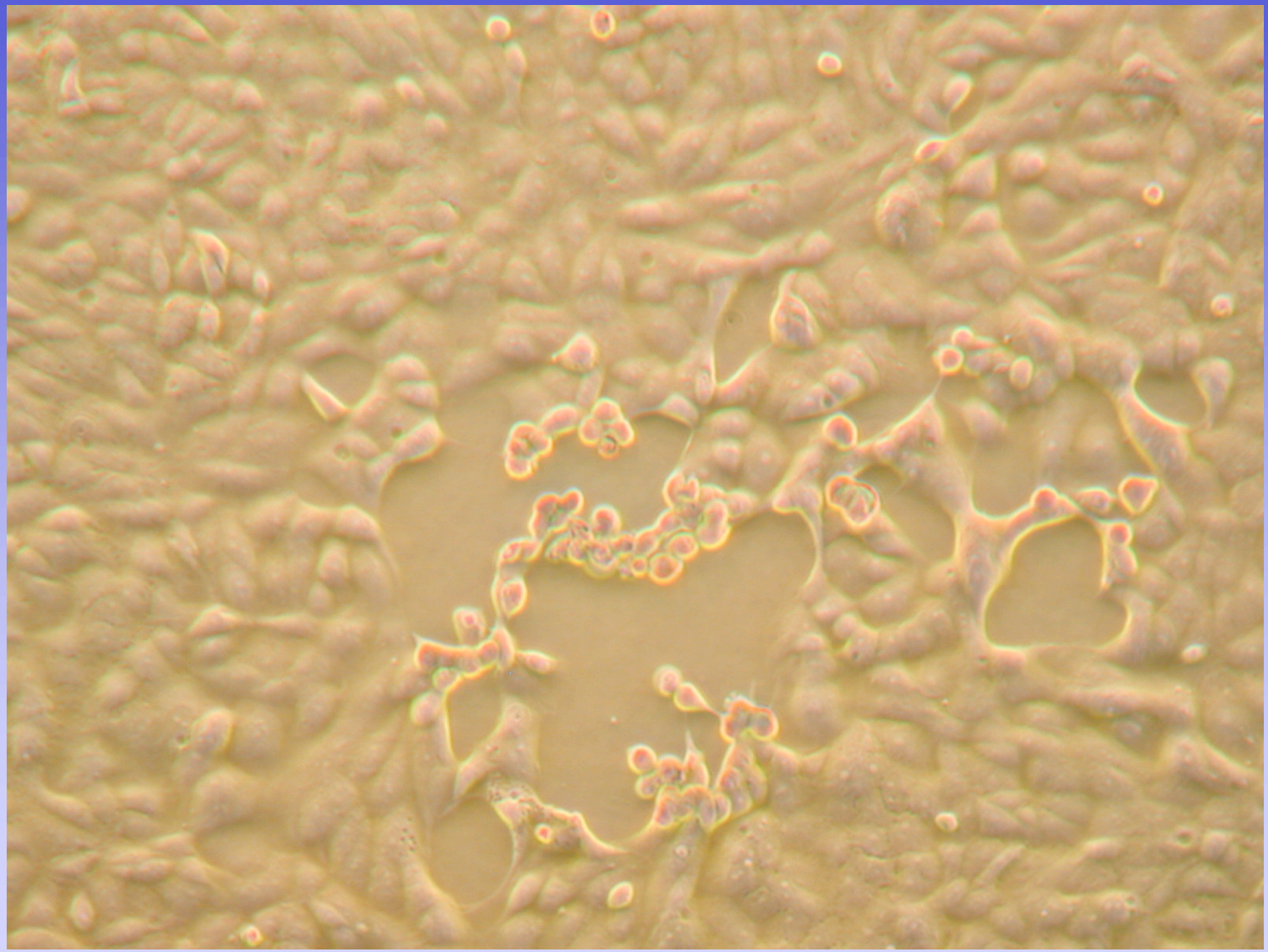


Coronavirus-infected Cells in BAL of SARS Patient

Thin Section EM

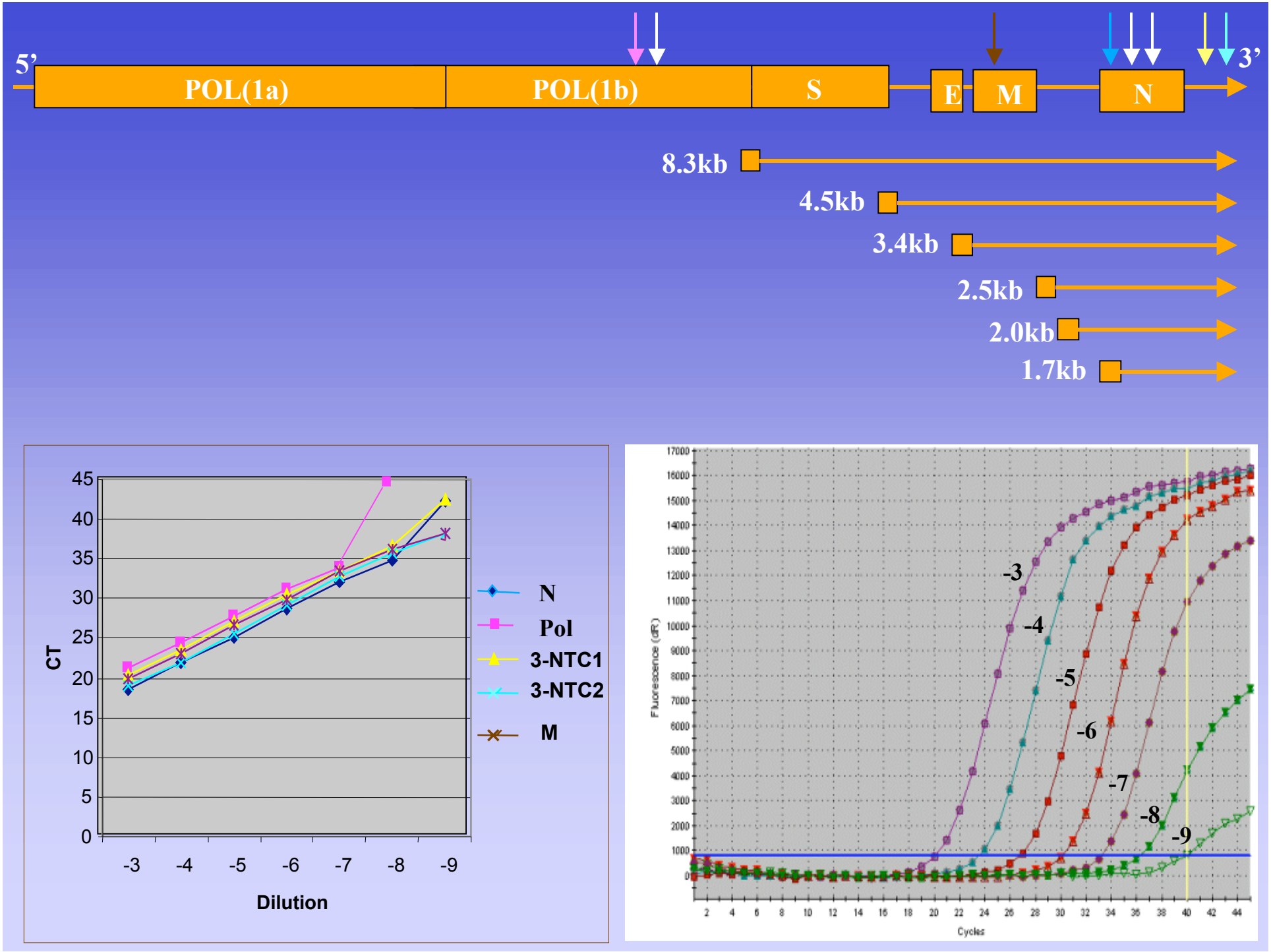


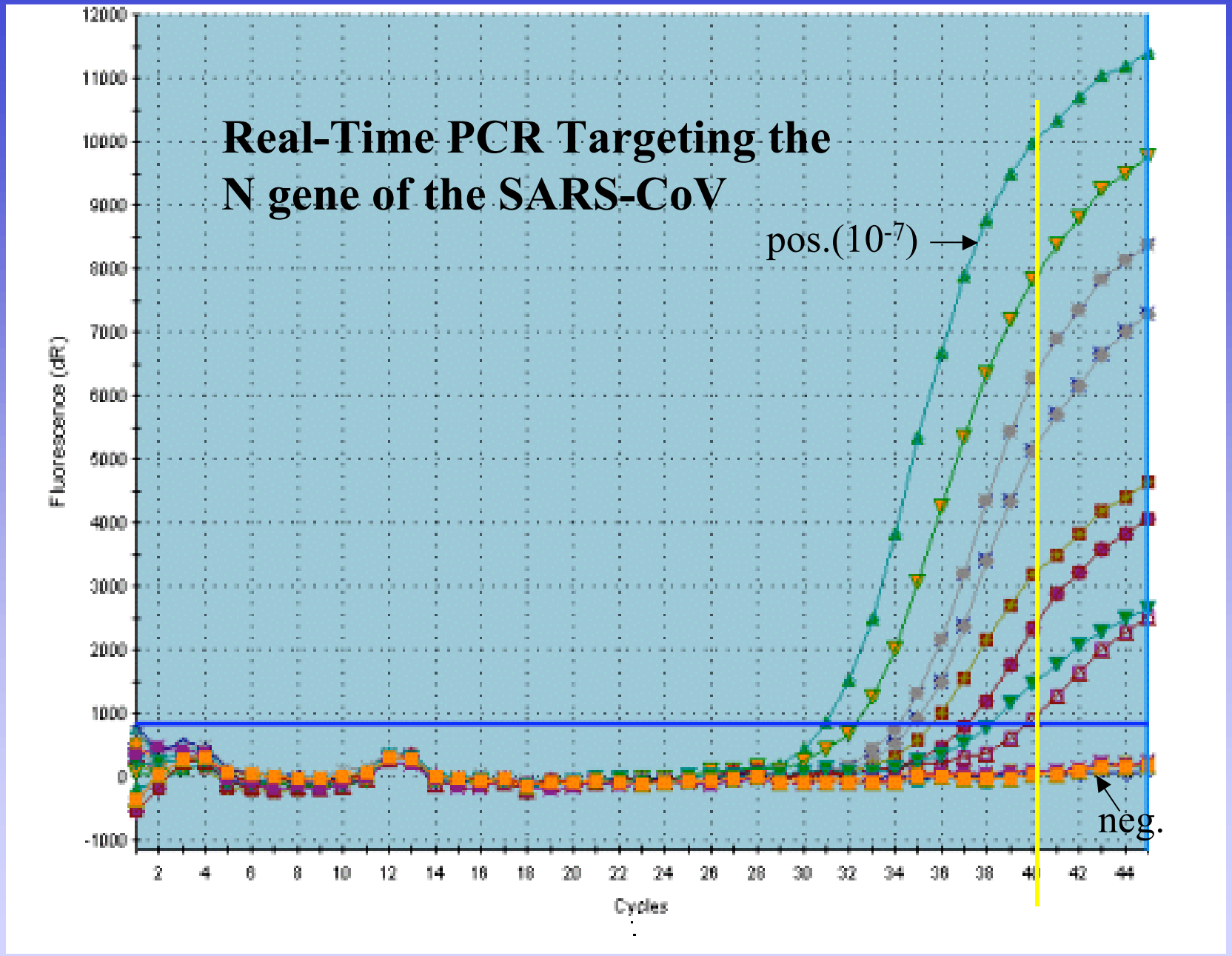
SARS-CoV Isolation in E6 Vero Cells



Real-Time RT-PCR Assays

- Polymerase region of CoV most conserved
  - Target for Artus commercial kit (Drosten et al. NEJM, 2003)
  - Target for CDC (Ksiazek et al. NEJM, 2003)
  - Target for U Hong Kong (Poon et al., Clin. Chem. 2003)
  - Several other groups
- Nucleocapsid gene most abundant
- Can be exquisitely sensitive, but contamination of specimens can be problematic.
  - Most assays can detect between 10-100 copies of target RNA
- Detection of RNA may not always be equated with infection.


5'
POL(1a)
POL(1b)
S
E
M
N
3'
8.3kb
4.5kb
3.4kb
2.5kb
2.0kb
1.7kb
CT
Dilution
N
Pol
3-NTC1
3-NTC2
M
Fluorescence (dR')
Cycles
-3
-4
-5
-6
-7
-8
-9


Real-Time PCR Targeting the
N gene of the SARS-CoV
pos.(10-7)
neg.
Fluorescence (dR')
Cycles
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
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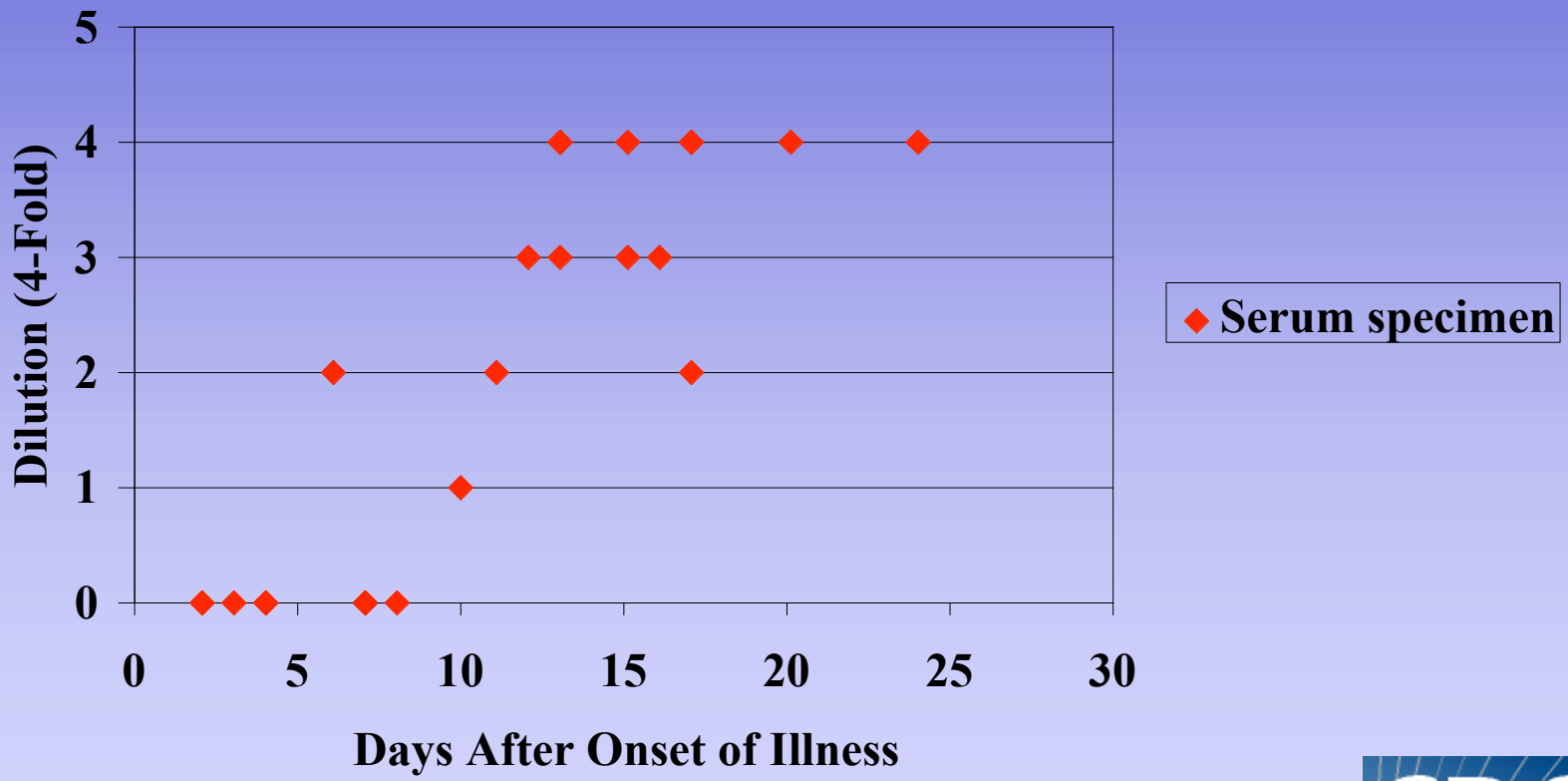
RT-PCR Findings

- Generally more successful early after onset of illness (2-5 days post disease onset), using oropharyngeal washes.
- Impression that the later in the course of illness, deeper respiratory specimens are most likely positive.
- Stool specimens are positive at about the same time as respiratory specimens (Lim et al), and viral load can be very high.
- Virus shedding in stool can occur after disease symptoms have resolved.
- Questions remain whether or not RT-PCR can be used as a single laboratory indicator of SARS-CoV infection.

EIA Antibody Response to SARS CoV

Ksiazek et al NEJM. 2003



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Serology Appears Specific

- Tests of sera with documented other human coronavirus infections (OC43 and 229E) show no reaction with the new novel coronavirus by either IFA or ELISA
- Tests of "normal" population of CDC and Emory U. blood donors demonstrate essentially no antibody in this population
  Similar tests in Hong Kong - same findings
- Sensitivity of tests might improve with better antigen (expressed) or different assay formats

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SARS CoV Testing 50 SARS Patients from Hong Kong

Peiris et al. http:// image.thelancet.com, 3/2003

Specimen	PCR	Serology	Either
Resp Sec	22/44		
Stool	10/18		
Serum		35/50	
Any			45/50*
ARI Controls		0/80	
Blood donors		0/200	

*4/5 antibody neg patients with serum collected <14 days after onset of illness

What Reagents and Tests Should Be Developed?

1. IgM Capture Assay – May provide early detection of infection

2. Monoclonal Ab – Useful for antigen detection in resp. secretions, tissue and reporters for capture EIAs, etc.

3. Recomb. Expressed Proteins – Useful in configuration of EIAs, Ab production.

4. Microarray Chips -- Detection of SARS-CoV in respiratory secretions, stool. ? sensitivity